Effects of Arsenic-, Platinum- and Gold-Containing Drugs on the Disposition of Exogenous Selenium in Rats

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Having found that the electrophilic model compound sulfobromophthalein markedly altered the fate of exogenous selenium in the body by reacting in vivo with nucleophilic selenium metabolites, the effects of metal-containing drugs with expected selenium reactivity were tested on biliary, urinary, and pulmonary excretion. Tissue distribution of selenium in selenite-injected rats was also examined. Coadministration with 

\[ {^{75}}Se \text{selenite} (10 \mu\text{mol/kg, iv}) \]

of the trypanosomicid arsenicals (100 \( \mu\text{mol/kg, iv} \)) trimelarsan (TMA) or melarsoprol (MAP), the antitumor cisplatin (25 \( \mu\text{mol/kg, iv} \)), or the antirheumatic gold sodium thiomalate (25 or 50 \( \mu\text{mol/kg, iv} \)) significantly altered the disposition of \( ^{75} \text{Se} \), whereas carboxplatin (100 \( \mu\text{mol/kg, iv} \)) did not produce such an effect. The most dramatic alterations included the \( \approx 20 \)-fold increase in the biliary excretion rate of selenium in response to TMA and MAP, the almost complete cessation of the exhalation of selenium as dimethyl selenide after administration of the arsenic- and gold-containing drugs, and the manifold accumulation of selenium in the blood plasma following gold injection. Direct chemical reaction of the drugs with nucleophilic selenite metabolites in the body may underlie these alterations. The tight coordination in time and extent observed between the biliary excretion of arsenic and selenium in rats receiving either of the arsenicals and selenite supports this hypothesis. However, attempts to detect selenium-containing biliary metabolites of TMA and MAP have failed, possibly owing to their instability. In summary, the arsenic-, platinum- and gold-containing drugs significantly influence the fate of exogenous selenium, whereby they may adversely affect the availability of this essential element for synthesis of selenoenzymes. Furthermore, the capability of TMA and MAP to enhance the biliary and total excretion of selenium renders these drugs significant candidates for antidotes in selenium intoxication.

Key Words: selenium; arsenic; melarsoprol; cisplatin; gold; biliary excretion.

The biological importance of selenium is at least 3-fold. First, selenium is an essential element because it forms the prosthetic group of some critical selenocysteine-containing enzymes, such as glutathione peroxidase, iodothyronine 5'-deiodinase, and thioredoxin reductase (Stadtman, 1996). Insufficient intake of selenium is thought to contribute to the pathogenesis of various degenerative and inflammatory diseases, aging and cancer (Lee et al., 1996; Levander, 1987; Nève, 1991). Second, sodium selenite is protective against a number of toxicants (Combs and Gray, 1998), including heavy metals (Diplock et al., 1986), arsenic (Hill, 1975) and chemical carcinogens (Schrauzer, 1992) in experimental animals, and it reduces the incidence of some tumors in humans (Clark et al., 1996; Han, 1993). Third, selenium has a significant toxic potential and its excessive intake or accidental ingestion has caused intoxications in humans and farm animals (Diplock, 1976; Levander, 1987; Mack, 1998).

Because of the importance of selenium for health, the disposition of this metalloid in the body as well as factors that influence the fate of selenium should be well understood. Selenite, an important exogenous source for endogenous selenium compounds, is known to be extensively biotransformed (Ganther, 1986; Ganther and Lawrence, 1997). It undergoes glutathione (GSH)-dependent reductions to form, consecutively, GS-Se-SG, GS-SeH, and hydrogen selenide (HSeH). The last is methylated sequentially, resulting in production of methylselenol (CH\(_3\)SeH), then the volatile and expirable dimethyl selenide (CH\(_3\)SeCH\(_3\)), and finally trimethylselenonium ion [(CH\(_3\))\(_3\)Se\(^{+}\)], which is excreted in urine. Selenide is also the precursor for selenocysteine and thus is needed for synthesis of selenoproteins (Stadtman, 1996). Additionally, selenide is also a toxic metabolite of selenite (Schrauzer, 1992), whereas methylselenol, is thought to be involved in both toxicity and anticarcinogenic activity (Ganther and Lawrence, 1997) of selenite.

Because numerous biotransformation and transport processes contribute to the disposition of selenium, the fate of this element in the body, and thus potentially its functions, can be subject to various influences. These can include changes in the availability of endogenous GSH (Gyurasics et al., 1998) and activity of methyltransferases (Hoffman and McConnell, 1987; Gyurasics et al., 1998). In addition, several exogenous chemicals, such as compounds of arsenic, antimony, and bismuth, influence disposition of selenite (Gregus et al., 1998a; Levander and Baumann, 1966). Most recently we have found that sulfobromophthalein, a cholephilic model compound with
electrophilic properties, when given to selenite-injected rats, increased the biliary excretion of selenium several-fold, while diminishing exhalation of dimethyl selenide (Gregus et al., 1998b; Gyurasics et al., 1998). These dramatic changes in selenium disposition were caused by in vivo reaction of the electrophilic sulfobromophthalein with the strongly nucleophilic metabolites of selenite (Gregus et al., 1998b). Based on this observation, we hypothesized that other electrophilic xenobiotics may also interfere with selenium metabolism by directly reacting with nucleophilic selenium metabolites in the body. Given the potentially significant implications of such interactions, we tested this hypothesis by investigating the effect of some therapeutically used chemicals containing an electrophilic atom on the fate of exogenous selenium. The drugs selected for this study included the antitrypanosomal arsenical drugs trimelarsan (TMA) and melarsoprol (MAP), the antineoplastic platinum coordination complexes cisplatin and carboplatin, and gold sodium thiomalate, which is used in the treatment of rheumatoid arthritis (Fig. 1). To determine whether these drugs interfere with the disposition of exogenous selenium, they were administered to rats injected with radioactive selenite and urinary, biliary, as well as pulmonary excretion, and tissue distribution of selenium were followed and compared to the excretion and distribution of the metalloid in selenite-injected, vehicle-treated rats.

**MATERIALS AND METHODS**

**Chemicals.** Sodium selenite (Na₂SeO₃·5H₂O) was purchased from Reanal (Budapest, Hungary) and sodium [³⁵Se]selenite (3 mCi/μmol) from Armerham International Plc (Little Chalfont, Buckinghamshire, England). TMA and Arsolab™ injection containing MAP in propylene glycol were generous gifts from Rhone-Poulenc Rorer (Vitry sur Seine, France) and Specia (Paris, France), respectively. We obtained cis-platinum(II)diammine dichloride (cisplatin) from Sigma Chemical Co., whereas carboplatin (Paraplatin™) and gold sodium thiomalate (Tauredon™) were products of Bristol-Myers Squibb Co. (Princeton, New Jersey) and Byk Gulden GmbH (Konstanz, Germany), respectively.

**Animal experiments.** Male Wistar rats (Charles River, Hungary, Budapest) weighing 260–320 g were used. The animals were kept at 22–25°C room temperature, at 55–65% relative air humidity, and on a 12-h light/dark cycle, and were provided with tap water and rat chow (type VRF1, Charles River Hungary) ad libitum.

The animal experiments for quantification of biliary, urinary, and pulmonary excretion as well as tissue distribution of selenium were performed as described (Gregus et al., 1998b). Briefly, the rats were hydrated by gavage of 30 ml/kg of saline containing 10 mM potassium chloride, anaesthetized by ip injection of a mixture of fentanyl, midazolam, and droperidol (0.045, 4.5, and 5.5 mg/kg, respectively) and their body temperature was maintained at 37°C by means of heating radiators. The left carotid artery was cannulated with polyethylene tubing (PE-50). A tracheotomy was performed in order to implant a Y-shaped trachea cannula through which the exhaled dimethyl selenide could be collected in an apparatus constructed for this purpose (Gregus et al., 1998b). Subsequently, the urinary bladder was exteriorized through a lower midline abdominal incision and the bile duct was cannulated through an upper midline abdominal incision as described above.

The rats, thus prepared, were administered 6 ml/kg 10% mannitol in saline via the carotid cannula to promote urine flow, and were subsequently injected with a subtoxic dose of sodium [³⁵Se]selenite (10 μmol/kg, 10 μCi/kg) into the left saphenous vein, then 1 min later with one of the drugs (at doses given in the figure legends) into the right saphenous vein. The drugs were dissolved in saline, except MAP, which was dissolved in propylene glycol. Control rats were given saline (3 ml/kg, iv) or propylene glycol (1.1 ml/kg, iv). Bile and urine samples were then collected in 20-min periods into preweighed 1.5-ml microcentrifuge tubes. To obtain urine, the urinary bladder was gently compressed manually when full and at the end of each collection period. To maintain urine flow at rates of 130–180 μl/kg/min, 3 ml/kg 10% mannitol in saline was injected via the carotid cannula every 20 min. Throughout the experiment (100 min), the air expired by the rat was continuously aspirated from the trachea cannula through 3 serially connected closed glass tubes containing 8 M HNO₃ solution (Gregus et al., 1998b). Nitric acid traps dimethyl selenide as nonvolatile dimethylhydroxyselenonium nitrate (Chalenger and North, 1934) and has been used to collect this volatile selenite metabolite exhaled by animals (Ganter et al., 1966; Hoffman and McConnell, 1987). At the end of each experiment, the rats were exsanguinated and tissue samples were removed for determination of [³⁵Se]selenium distribution.

**Analysis.** The amount of [³⁵Se]selenium in bile, urine, the HNO₃ solutions that entrapped the exhaled dimethyl selenide, and the weighed tissue samples was determined by measuring the radioactivity of the collected biological samples in a well-type γ scintillation counter (Type NK-350, Gamma Works, Budapest). Standard solutions containing known amounts of [³⁵Se]selenite were also counted to calculate the dosimetry. Arsenic in the bile samples collected from the rats injected with TMA or MAP was quantified by inductively coupled plasma atomic emission spectrometry using a Spectrofame ICP spectrometer (Spectro Analytical Instruments, Kleve, Germany). In the bile of rats not injected with arsenicals, the concentration of arsenic was below the detection limit (0.05 μg/ml).

**Statistics.** Data were analyzed by ANOVA followed by Duncan’s test with p < 0.05 as the level of significance.

**RESULTS**

**Effect of Arsenical Drugs on Selenium Disposition**

Excretion and distribution of exogenous selenium in rats injected with sodium selenite and TMA or MAP is presented in Figure 2. Both arsenicals dramatically increased the biliary excretion of selenium (Fig. 2, upper left). Whereas the vehicle-injected rats excreted selenium into bile at a steady rate of approximately 3.5 nmol/kg/min, biliary selenium output in rats given TMA or MAP peaked at rates exceeding 50 nmol/kg/min early after injection of the drugs. Later, the biliary selenium excretion in the treated rats gradually declined, but even at
80–100 min after administration of either arsenical, it exceeded the control rate more than 5-fold. The cumulative biliary excretion of selenium was enhanced by TMA and MAP 12- and 10-fold, respectively (Fig. 2, upper right).

In contrast to the biliary excretion, the urinary excretion of selenium was differentially and little affected by the 2 arsenical drugs (Fig. 2, lower left). TMA increased the output of selenium into urine by 60% early after its injection, but not at later time points. In contrast, MAP did not influence the urinary selenium excretion initially, but later lowered it by 50 to 64%. The cumulative urinary excretion of selenium was increased by TMA (45%) and was diminished insignificantly by MAP (Fig. 2, upper right).

Exhalation of dimethyl selenide, which was quantitatively the most significant route for selenium excretion in control rats, was almost completely abolished by the arsenicals, as the cumulative excretion of selenium by the exhaled air was lowered by TMA and MAP to 6% and 2% of the control, respectively (Fig. 2, upper right). In spite of this, the cumulative total (i.e., biliary + urinary + pulmonary) excretion of selenium was increased 2.8-fold by TMA and 2.1-fold by MAP (Fig. 2, upper right).

Both arsenic-containing drugs altered the tissue distribution of selenium, albeit not uniformly (Fig. 2, lower right). TMA and MAP alike diminished the concentration of selenium in blood and liver. TMA, but not MAP, significantly increased the renal selenium levels, and decreased selenium content in plasma, muscle, testis, and brain. Plasma selenium concentrations were higher in the rats receiving MAP than in the rats given the vehicle.

The striking arsenical-induced enhancement in the biliary excretion of selenium was further analyzed. Figure 3 depicts...
the time courses of the biliary excretion of arsenic and selenium in rats injected with sodium selenite plus TMA or MAP. Trimelarsan or melarsoprol (100 μmol/kg, iv) were injected into anesthetized, bile duct-cannulated rats 1 min after administration of sodium [75 Se]selenite (10 μmol/kg, iv), and bile was collected thereafter at 20-min intervals for 100 min for quantification of total arsenic and selenium. Symbols represent means ± SEM of 5 rats.

FIG. 3. Simultaneous biliary excretion of arsenic and selenium in rats injected with selenite and trimelarsan or melarsoprol. Trimelarsan or melarsoprol (100 μmol/kg, iv) were injected into anesthetized, bile duct-cannulated rats 1 min after administration of sodium [75 Se]selenite (10 μmol/kg, iv), and bile was collected thereafter at 20-min intervals for 100 min for quantification of total arsenic and selenium. Symbols represent means ± SEM of 5 rats.

FIG. 4. Effect of selenite on the biliary excretion of arsenic in rats injected with trimelarsan (TMA) or melarsoprol (MAP). TMA or MAP (100 μmol/kg, iv) was injected into anesthetized, bile duct-cannulated rats 1 min after administration of sodium selenite (10 μmol/kg, iv), and bile was collected thereafter at 20-min intervals for 100 min for quantification of total arsenic. Symbols represent means ± SEM of 5 rats. Asterisks indicate arsenic excretion rates of the selenite plus arsenical-injected rats significantly different (p < 0.05) from the respective rates of the arsenical-injected rats.

In order to find out whether selenite also increases the biliary excretion of arsenic, output of arsenic into bile in rats given either arsenical alone or arsenical in combination with selenite were compared. Figure 4 indicates that coadministration of selenite did not influence significantly the early occurrence of maximal arsenic excretion rates either in rats injected with TMA (left) or in rats given MAP (right); however, later selenite plus arsenical-injected rats excreted less arsenic into bile than those receiving TMA or MAP alone.

Effect of Platinum-Containing Drugs on Selenium Disposition

Figure 5 depicts the effects of cisplatin and carboplatin on the excretion and distribution of selenium. Both platinum coordination complexes enhanced the biliary excretion of selenium (Fig. 5, upper left); however, cisplatin was more effective in this respect despite the fact that it was given at a lower dose than carboplatin. For example, while cisplatin more than doubled the maximal biliary excretion rate of selenium and elevated the cumulative selenium output into bile by 81%, carboplatin increased these values only by 63 and 40%, respectively (Fig. 5, upper left and right).

Neither platinum-containing compound significantly influenced the course of urinary output (Fig. 5, lower left) or the cumulative urinary, respiratory as well as total (bile + urine + exhaled air) excretion of selenium (Fig. 5, upper right). As shown in the right lower panel of Figure 5, the tissue distribution of selenium was altered moderately by cisplatin, resulting in higher selenium concentrations in the liver, kidney, and plasma, and lower selenium levels in testis and brain than in
the saline-injected controls. In contrast, distribution of selenium was not influenced significantly by carboplatin.

**Effect of Gold Sodium Thiomalate on Selenium Disposition**

Figure 6 presents the effect of gold sodium thiomalate, given in 25 or 50 μmol/kg intravenous doses, on the disposition of exogenous selenium in sodium selenite-injected rats. The gold compound did not enhance selenium excretion into bile; moreover, it significantly depressed the initial biliary excretion of selenium when the drug was administered at the higher dose (Fig. 6, upper left). The course of urinary selenium excretion remained unchanged in gold-injected rats compared to the saline-injected controls, except after 60 min, when the gold compound given at the higher dose significantly depressed selenium excretion into urine (Fig. 6, lower left).

While administration of gold sodium thiomalate did not influence the cumulative biliary and urinary excretion of selenium, it depressed the exhalation of selenium markedly and in a dose-dependent fashion (Fig. 6, upper right). The 82 and 95% decreases in selenium exhalation in the rats injected with 25 and 50 μmol/kg gold, respectively, account for the 52 and 65% corresponding decreases in the total cumulative selenium excretion in these animals (Fig. 6, upper right).

The tissue distribution of selenium was significantly influenced in gold-treated rats compared to controls (Fig. 6, lower right). The animals injected with gold exhibited approximately 5- and 10-fold increases in the blood and plasma concentrations of selenium, respectively. There were more moderate elevations in the renal and testicular selenium levels, whereas the hepatic selenium content was decreased. Selenium distribution to the muscle and brain, however, was not altered by the gold compound.
DISCUSSION

Selenite metabolites, such as hydrogen selenide and methylselenol, are strong nucleophiles and thus exhibit high affinity to electrophiles (Ganther and Kraus, 1984). In this respect these selenite metabolites are similar to thiols, and therefore compounds that react with thiols (e.g., GSH) are expected to react also with selenols, both in vitro and in vivo. Indeed, in selenite-injected rats the electrophilic sulfobromophthalein is conjugated not only with endogenous GSH but also with exogenous selenite-derived hydrogen selenide and methylselenol (Gregus et al., 1998b), whereby it influences the disposition of selenium markedly.

The melaminophenyl arsenical drugs TMA and MAP also readily form a GSH conjugate, tentatively identified as melarsene-diglutathione, and are rapidly excreted into bile largely as this conjugate (Gregus and Gyurasics, 2000). This, as well as earlier observations indicating that the inorganic arsenical arsenite and arsenate markedly enhance the biliary excretion of exogenous selenium (Gregus et al., 1998a; Levander and Baumann, 1966), suggested that TMA and MAP may also interact with selenium in the body. Although such interaction has been hypothesized to produce adverse clinical outcome (Golden, 1992), its occurrence has remained unproven. This study was aimed primarily at exploring the possibility of the interaction of arsenical drugs with selenium by studying the effects of TMA and MAP on the biliary, urinary, and expiratory excretion as well as tissue distribution of selenium in selenite-injected rats. We have performed similar studies with platinum- and gold-containing drugs, which are also electrophilic.

FIG. 6. Effect of gold sodium thiomalate (gold) on the biliary, urinary, as well as pulmonary excretion and tissue distribution of selenium. Gold (25 and 50 μmol/kg, iv) or saline (3 ml/kg, iv) was injected into anesthetized, bile duct-cannulated rats 1 min after administration of sodium [75Se]selenite (10 μmol/kg, iv). Thereafter, bile and urine were collected periodically and [75Se]dimethyl selenide from the expired air was collected continuously for 100 min when the tissue samples were removed. Symbols and bars represent means ± SEM of 5–6 rats. Asterisks indicate significant difference (p < 0.05) from the respective value of the saline-injected rats.
1998; Sasada et al., 1999) has been documented, information on their effects on the fate of exogenous selenium is limited (Vermeulen et al., 1993).

The present work demonstrates that the arsenic-, platinum- and gold-containing drugs significantly alter the excretion and distribution of exogenous selenium in rats. The most striking changes included the several-fold increase in biliary excretion of selenium caused by the arsenicals (Fig. 2, upper left), the almost complete cessation of selenium exhalation induced by the arsenic- and gold-containing drugs (Figs. 2 and 6, upper right), and the marked accumulation of selenium in the blood following administration of the gold compound (Fig. 6, lower right).

Enhancement of selenium excretion into bile by the arsenicals was not unexpected. Although sodium arseniate, an organic arsenical containing pentavalent arsenic, did not increase the biliary selenium output (Levander and Baumann, 1996), the inorganic pentavalent arsenate, which is readily reduced in vivo and is excreted into bile exclusively in trivalent forms (Gregus et al., in press), enhanced the maximal rate of biliary selenium excretion more than 40-fold (Gregus et al., 1998a). The trivalent arsenite was also highly effective in stimulating the hepatobiliary transport of selenium in selenite-injected rats (Gregus et al., 1998a; Levander and Baumann, 1966). The present study demonstrates that the trivalent organic arsenicals TMA and MAP were equally effective with arsenite in elevating the maximal rate of selenium output in bile. However, while inorganic arsenicals and selenium mutually increased the biliary excretion of each other (Gregus et al., 1998a; Levander and Baumann, 1966), selenite failed to enhance the hepatobiliary transport of arsenic in TMA- or MAP-injected rats (Fig. 4). Compared to the arsenicals, the platinum-containing drugs produced only modest increases in biliary selenium excretion (Fig. 5).

Diverse mechanisms may underlie the xenobiotic-induced increases in the hepatobiliary transport of exogenous selenium. It has been demonstrated that inhibition of selenium methylation by pretreatment with periodate-oxidized adenosine doubles the rate of biliary selenium excretion in selenite-injected rats (Gyurasics et al., 1998). Because the magnitude of this effect is much smaller than that of TMA or MAP, the enhancement of selenium excretion into bile by these arsenicals cannot be ascribed to their possible inhibitory action on formation of methylated selenite metabolites. Such interaction is unlikely to be responsible for even the moderate increase in the biliary excretion of selenium in response to the platinum compounds, because these drugs did not diminish exhalation of dimethyl selenide significantly (Fig. 5, upper right), indicating that they did not interfere with selenium methylation.

It has long been hypothesized that the inorganic arsenicals augment the biliary excretion of selenium by forming a conjugate with selenium that is rapidly excreted into bile (Levander and Baumann, 1966). Although such conjugates have not been demonstrated, due to their purported instability, the tightly coordinated hepatobiliary transport of selenium and arsenic in rats injected with selenite plus arsenite or arsenate (Gregus et al., 1998a) supports the hypothesis that they form common compounds in the liver that are transported into bile. The close correlation in time and magnitude between the biliary excretion of selenium and arsenic is also evident in rats injected with selenite plus TMA or MAP (Fig. 3). This finding may indicate that TMA and MAP form one or more common cholephilic metabolites with selenite in vitro. Our recent finding that the monothiol GSH reacts with these arsenicals both in vitro and in vivo by replacing the dithiol moiety of these drugs and forming a di-GSH conjugate (Gregus and Gyurasics, 2000) makes it feasible that the selenol metabolites of selenite are also capable of such substitution reaction. The common selenium containing biliary metabolite of TMA and MAP, however, should retain cholephilic properties, i.e. anionic charge(s) and a molecular weight above 350. Therefore, it can be speculated that, in the common biliary metabolite, one GSH moiety of the di-GSH conjugate formed from TMA or MAP may be substituted by a selenol moiety (e.g., CH3-SeH), whereas the other GSH moiety is retained to ensure the cholephilic property. We have made attempts to demonstrate the existence of selenium-containing biliary metabolites of TMA and MAP in rats injected with [75Se]selenite plus either of these arsenicals. For this purpose, we combined an HPLC separation procedure that had been successfully used for identification of the biliary metabolites of TMA and MAP (Gregus and Gyurasics, 2000) with simultaneous monitoring of the HPLC effluent with absorbance and radioactivity detectors, a strategy successfully employed for identification of selenium-containing biliary metabolites of sulfobromophthalein (Gregus et al., 1998b). Unfortunately, the several biliary selenium compounds detected with the radioactivity detector were eluted in a highly irreproducible fashion and typically not simultaneously with any of the TMA and MAP metabolites that were detected by the absorbance detector. This observation suggests that if selenium-containing arsenical metabolites are indeed formed in the liver, they do not survive long after their hepatobiliary transport. Instability of arsenic-GSH complexes at an alkaline pH that occurs in bile has been demonstrated (Delnomdedieu et al., 1994) and is thought to contribute to the large increase in biliary GSH output evoked by inorganic arsenicals (Gyurasics et al., 1991a,b). A similar event may befall the hypothesized selenium-containing metabolites of TMA and MAP in the bile.

Cholephilic selenium-containing metabolites may be formed also in selenite-injected rats receiving the platinum drugs. Cisplatin, and much less so, carboplatin, enters into substitution reaction with GSH in vitro (Berners-Price and Kuchel, 1990; Dedon and Borch, 1987) and a bis-(glutathionyl) platinum complex is also formed in cisplatin-exposed cells (Ishikawa and Ali-Osman, 1993). Substitution of one GSH with a selenol in such a complex in the liver and subsequent hepatobiliary transport of this ternary complex could explain the
slight increases in biliary selenium output in rats receiving selenite and the platinum-containing drugs. Nevertheless, the slow biliary excretion of these drugs in rats (cisplatin ~ 1%, carboplatin ~ 0.25% of dose in 2 h; Siddik et al., 1987), compared to the extremely rapid hepatobiliary transport of TMA and MAP metabolites (more than 50% of dose in 100 min; Gregus and Gyurascus, 2000), likely accounts for the observation that the platinum coordination complexes are much inferior to the melanophenyl arsenicals in augmenting the biliary excretion of selenium. Gold is also transported into bile at extremely low rates (Gregus and Klaassen, 1986; Masarella and Pearlman, 1987) and fails to promote the biliary excretion of selenium (Fig. 6) despite its capability to form a bisglutathione complex (see below).

Dramatic diminution of selenium exhalation was brought about by coadministration of TMA and MAP, or gold sodium thiomalate with selenite (Figs. 2 and 5). Arsenite and arsenate also markedly reduces the pulmonary excretion of selenium in selenite-exposed rats (Foster et al., 1986; Olson et al., 1963). Because the exhaled selenium compound is dimethyl selenide, diminished formation of this volatile product is the most likely mechanism involved. Indeed, impairment of selenium methyl-ation caused by treatment with periodate-oxidized adenosine or depletion of vitamin B12 lowers selenium exhalation significantly (Hoffman and McConnell, 1987; Chen and Whanger, 1993). Sodium arsenite potently inhibited formation of dimethyl selenide from selenite in the microsomal fraction of rat liver (Hsieh and Ganther, 1977), purportedly by blocking thiol-methyltransferase that is thought to catalyze methylation of hydrogen selenide to methylselenol and the latter to dimethyl selenide. Whether TMA, MAP, or the gold compound inhibits this methyltransferase is not known. Nevertheless, it is also possible that reaction of these drugs with hydrogen selenide and methylselenol accounts for the fall in selenium exhalation, because these reactions would consume the aforementioned metabolites that are precursors of dimethyl selenide. Sulfobromophthalein that reacts with these selenite metabolites is also significantly diminishes selenium exhalation (Gregus et al., 1998b).

Except for carboplatin, which is much less reactive with nucleophiles than cisplatin (Dedon and Borch, 1987), the drugs studied influenced the tissue distribution of selenium. However, by far the largest relative change was observed in gold-injected rats, which exhibited several-fold accumulation of selenium in the circulation. On estimating the selenium concentration in red blood cells, based on the levels of selenium in the whole blood and plasma and a hematocrit value of 0.45, it can be concluded that the increase in blood-borne selenium following gold treatment resulted almost exclusively from accumulation of the metalloid in the plasma and not in the erythrocytes. After administration of gold sodium thiomalate to rats, practically all gold in the circulation is found in the plasma (Walz et al., 1979). It has been proposed that endogen-ous thiols, including GSH, rapidly displace gold from its original complex. This substitution reaction produces new complexes, such as bis(glutathione)-gold(I) complex or Au(GS)₂⁺ (Chaudiere and Tappel, 1984; Shaw et al., 1990), which in turn react with albumin at its cysteine-24, a very high-affinity gold-binding site (Shaw et al., 1990). Thus, the thiol-gold-albumin complex is formed, such as GS-Au-S-albumin, which explains the extensive binding of gold to plasma albumin. It appears likely that the small-molecular-weight thiol in this complex could be easily substituted with a selenol, whereby the gold would bind the selenium to albumin (e.g., R-Se-Au-S-albumin) and would cause accumulation of this metalloid in the blood plasma. Other changes in selenium distribution observed in response to administration of the metal-containing drugs might also result directly from in vivo chemical reactions between the drugs and the nucleophilic selenite metabolites. However, the decreased selenium levels in the blood, liver, muscle, testis, and brain in rats injected with the arsenicals, as compared to the vehicle-dosed rats (Fig. 1), may also be secondary to the great TMA- and MAP-induced increases in the biliary and total selenium excretion and/or to the suppression of selenium methylation.

With regard to the possible implications of our findings, it is worth noting that the drugs included in this study, except for the arsenicals, have been demonstrated to lower the activity of the selenoenzyme GSH peroxidase after prolonged treatment. For example, the GSH peroxidase activity was significantly diminished in the monocytes of gold-treated rheumatoid arthritis patients (Reglinski et al., 1997), in the kidneys of the gold-treated rats (Baker et al., 1985; Dillard et al., 1987), and in the renal mitochondria of cisplatin-treated rats (Sugiyama et al., 1989). As these drugs diminish the activity of selenoenzymes, both after acute administration to animals (Smith et al., 1999) and in vitro (Berry et al., 1991; Gromer et al., 1998; Hill et al., 1997; Sasada et al., 1999), their direct inhibitory action, presumably via covalent reaction with the selenol group of the selenocysteine residue (Chaudiere and Tappel, 1984), is one mechanism whereby they impair the activity of GSH peroxidase and possibly other selenoenzymes, following prolonged exposure. In addition, several findings suggest that these drugs also impair the utilization of selenium for selenoprotein synthesis. For example, gold treatment produced a more signifi-cant decline in tissue GSH peroxidase activity in rats with suboptimal selenium intake than in rats with optimal intake (Baker et al., 1985) and selenite cotreatment prevented cisplatin-induced decline in GSH peroxidase activity in the renal mitochondria of rats (Sugiyama et al., 1989). Furthermore, repeated courses of cisplatin treatments caused progressive declines of plasma selenium levels in patients (Vernie et al., 1988).

The present study indicates that the arsenic-, platinum- and gold-containing drugs interfere with the disposition of exoge-nous selenium by altering its distribution, methylation, and/or excretion. The known reactivities of these drugs with thiols, selenium compounds, and selenoenzymes suggest that this
interaction involves covalent reaction of the drugs in the body with nucleophilic metabolites of selenite. Such reactions alone can diminish availability of exogenous selenium for synthesis of selenoenzymes. The arsenicals, in addition, also dramatically increase the biliary excretion of selenium and thus may cause loss of selenium from the body. Impairment of selenoprotein synthesis and/or activity may contribute to the numerous toxic effects of arsenic-, platinum- and gold-containing drugs. It has been hypothesized on theoretical grounds recently that the encephalopathy associated with MAP treatment of African trypanosomiasis arises in patients who are selenium-deficient (Golden, 1992). The findings presented here indicate that MAP has the potential to adversely affect selenium homeostasis in rats and to counter uptake of this essential element into the brain. Thus, until information on the effect of MAP on selenium homeostasis in humans becomes available, the suggestion that MAP treatment should be performed with prior and concomitant selenium supplementation (Golden, 1992) is worthy of following. In addition, the present results also raise the possibility that the melaminophenyl arsenical drugs could be clinically effective antidotes in acute selenium intoxication.

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